THE LIFE-CYCLE OF PORPHYRA TENERA IN VITRO

HIDEO IWASAKI [1]

Haskins Laboratories, 305 East 43rd Street, New York 17, N. Y.

Cultivation of the red sea-weed *Porphyra tenera* was started in Japan several centuries ago. It is now the largest industrial cultivation of any marine product. Despite this success, more knowledge of the life-cycle and innate potencies of *Porphyra* is needed to improve methods of cultivation—to bring them under a control comparable to that achieved in land agriculture. At present this goal is unrealizable to its fullest extent. Mass-scale use of artificial fertilizers on 50 square miles of bays is uneconomical. But improvements in production and control of seeding, genetic improvement of the plant in respect to greater production and resistance to parasites, and perhaps extension of the growth period of the thallus, seem attainable goals.

Several obstacles have slowed research on the life-cycle and potencies of *Porphyra*. Until a few years ago only one part of the life-cycle was known: mysteriously, the bays abound in monospores in the autumn; these monospores, collected on bamboo or cord nets, develop into the edible, leafy thallus which is periodically harvested until March, when it fruits and disintegrates while producing carpospores. What was happening to the carpospores, and the origin of the monospores, were unknown. Those mysteries were solved after Drew (1949) discovered that the carpospores of another species, *Porphyra umbilicalis,* germinated into a filament which, in enriched sea water, produced a flimsy, sickly mat. The fact that the germ tubes produced by the carpospores are very similar to those of fungal spores, and that the older filaments of the mat are generally abnormal in appearance suggested to her that a specific host or a special substrate are needed for normal growth. Indeed several molluscan shells and even egg shells proved an excellent substrate. The filamentous thallus grows well in the shells, forming colonies identical with *Conchocelis rosea*; *C. rosea* is obviously merely a phase of the life-cycle of *Porphyra*. Kurogi (1953) and Tseng and Chang (1954) found that the carpospores of *Porphyra tenera* behaved similarly. Kurogi (1953) studied the growth of the carpospores of *Porphyra umbilicalis prox., P. suborbiculata, P. pseudolinearis* and *P. tenera*; these form "*Conchocelis*" colonies in the shells which can hardly be told from one another. In Kurogi's cultures the "*Conchocelis*" phase cultured on glass slides produced monosporangiate branches but not free monospores. However, from *Conchocelis* in oyster shells Kurogi (1953) obtained monospores which produced germlings of the leafy thallus.

The complete life-cycle of *Porphyra* was now known. This discovery renewed interest in the biology of *Porphyra,* especially the conditions for growth (Iwasaki and Matsudaira, 1958) and production of monospores (Kurogi and Hirano, 1956).

[1] Present Address: Tohoku University, Faculty of Agriculture, Department of Fisheries, Sendai, Japan.

Obstacles to speedy progress were: (*a*) inability to grow the *Conchocelis* phase outside of the shells in free conditions; and (*b*) inability to cultivate in the laboratory, out of season, the two growth phases of *Porphyra* which in nature are strictly seasonal (autumn-winter for the thallus phase and spring-summer for the *Conchocelis* phase).

As mentioned, Drew and Kurogi had grown the *Conchocelis* phase in enriched sea water on glass slides. Although the growth of *Conchocelis* was poor, Drew (1954) mentioned (p. 193) ". . . . that such free-living filamentous growths can be maintained and continue to grow indefinitely provided the culture solution is renewed regularly"; this seemed promising. Drew obtained with *P. umbilicalis* only filamentous *Conchocelis* growth on glass slides and no monosporangia were formed, while the four species of *Porphyra* (including *P. umbilicalis prox.*) studied by Kurogi produced monosporangia. The discrepancy between these results implied that good growth and fruiting of the *Conchocelis* phase in the free-living conditions might be obtained under different cultural conditions and with better media. Another reason for trying again to grow the *Conchocelis* phase of *P. tenera in vitro* was the success of Hollenberg (1958) in obtaining on glass slides in liquid media minute filamentous *Conchocelis*-like plants of *P. perforata* which produced "sporangia branchlets" and fertile "conchospores."

Preliminary Experiments

The original materials brought from Japan were a few sterilized oyster shells which had been inoculated in March, 1959, with carpospores produced by natural-grown thalli. Colonies of *Conchocelis* developed normally in the shells kept in Woods Hole sea water enriched with nitrate, phosphate, and EDTA, (medium SWI, Table I) indicating the suitability of Atlantic sea water for *Porphyra tenera.*

Table I

Enriched sea water media

	SWI	SWII
Filtered sea water	1000 ml.	1000 ml.
KNO_3	72.2 mg. (=10 mg. N)	72.2 mg. (=10 mg. N)
KH_2PO_4	8.8 mg. (=2 mg. P)	4.5 mg. (=1 mg. P)
Na_2-glycerophosphate $.5H_2O$		10.5 mg. (=1 mg. P)
Fe-EDTA (1:1 chelation)	0.5 mg. (as Fe)	0.5 mg. (as Fe)
"Tris" buffer*	500 mg.	500 mg.
pH	8.0–8.2	8.0–8.2

* Tris (hydroxymethyl) amino methane (Sigma Company).

The nutrient solution was changed fortnightly; the shells were periodically cleaned with cotton to eliminate epiphytic diatom growth and kept in subdued, continuous fluorescent light [2] (10–30 foot-candles) at 13–15° C. In September, 1959, some shells were broken in pieces and thin flakes containing one *Conchocelis* colony were thoroughly wiped clean of epiphytes with cotton and also by repeated dipping into 1.5% agarized enriched sea water containing antibiotics. The flakes were

[2] "Cool white."

then inoculated into various artificial marine media and in enriched sea water, and kept in continuous subdued light at 13–15° C. At the end of December, 1959, in two tubes of medium ASP_2NTA and in a tube of SWI + 5 μg.% indolacetic acid, a few young thalli appeared at the bottom of the test tube and on the shell flakes. Simultaneously in two tubes of SWI, tufts of free *Conchocelis* were growing out from the shell flakes. Later on (February–April, 1960), free *Conchocelis* colonies, attached to the bottom of the test tube or to the shells, appeared in the two ASP_2NTA tubes and the SWI + IAA. The young thalli and the free *Conchocelis* employed in the subsequent experiments were derived from these 5 original cultures, which are unialgal, but accompanied by bacteria and yeasts. Microbial contamination, though permanent, was minimal in all the media employed because of the lack of organic substrates and because aseptic techniques were employed throughout.

In Vitro Culture of Free-Living Conchocelis Phase

Origin of free Conchocelis

Strains of free-living *Conchocelis* were obtained in several ways: (1) from the free *Conchocelis* growing out of the shell flakes in SWI medium; (2) directly from carpospores released by mature thalli collected in Japan, shipped to New York (March, 1960), and germinated in liquid media; (3) from carpospores produced by thalli grown in artificial media *in vitro*.

At first, on the assumption that a substrate might be somehow advantageous to the *Conchocelis* phase, tufts of *Conchocelis* filaments, cut from the free growth on shell flakes in SWI, were transferred into biphasic media. To simulate the conditions in shells, the solid phase (10 ml. ASPI medium + agar 1.5%) was enriched with 0.1% $CaCO_3$, 0.01% chondroitin, or both; the liquid phase consisted of 5 ml. either of ASP7 or SWI; the *Conchocelis* tufts were implanted in the agar at the interphase. All these combinations allowed good growth at 13–15° C. and continuous subdued light and at 18–20° C. and 10 hours daily. In 2–3 months, from an initial tuft 1 mm. in length, spherical colonies of 0.5–1 cm. were obtained; later, new colonies formed at the interphase or on the glass wall. Growth was almost entirely in the liquid phase and in all the different combinations, indicating that a solid substratum rich in $CaCO_3$ or protein is unnecessary. Further experiments were done in liquid media to determine the best cultural conditions for free growth in liquid media.

Once some of these conditions were known, it became possible to germinate directly in liquid media carpospores collected from thalli grown either in nature or *in vitro*. Thalli of *P. tenera* collected in Matsukawa-ura inlet were shipped to New York in March, 1960. Following the method suggested by Professor Y. Yamada of Hokkaido University, the thalli were put between pads of absorbent cotton wet with sea water and shipped in Thermos bottles; this method avoids rotting and gives good survival. Upon their arrival in New York, the thalli were placed in enriched sea water and produced carpospores. The collected carpospores were washed several times in sterile sea water by means of capillary pipettes, and 3–5 carpospores were inoculated in test tubes containing 10 ml. of 3 types of enriched sea water (ASW8, SWI, SWII) and 9 artificial media (ASM, ASP1,

TABLE II

Artificial media composition (w./v.)

	ASP1	ASP2(NTA)	ASP6	ASP7	ASP12(NTA)*
Distilled water	100 ml.	100 ml.	100 ml.	100 ml.	100 ml.
NaCl	2.4 g.	1.8 g.	2.4 g.	2.5 g.	2.8 g.
$MgSO_4 \cdot 7H_2O$	0.6 g.	0.5 g.	0.8 g.	0.9 g.	0.7 g.
$MgCl_2 \cdot 6H_2O$	0.45 g.				0.4 g.
KCl	0.06 g.	0.06 g.	0.07 g.	0.07 g.	0.07 g.
Ca (as Cl^-)	40 mg.	10 mg.	15 mg.	30 mg.	40 mg.
$NaNO_3$	10 mg.	5 mg.	30 mg.	5 mg.	10 mg.
K_2HPO_4	2 mg.	0.5 mg.			
K_3PO_4					1.0 mg.
Na_2-glycerophosphate			10 mg.	2 mg.	1.0 mg.
$Na_2SiO_3 \cdot 9H_2O$	2.5 mg.	15 mg.	7 mg.	7 mg.	15 mg.
Na_2CO_3		3 mg.			
Fe (as Cl)		0.05 mg.			
B_{12}	0.02 μg.	0.02 μg.	0.05 μg.	0.1 μg.	0.02 μg.
Biotin					0.1 μg.
Thiamine					10 μg.
Vitamin mix 8**	0.05 ml.		0.1 ml.		
Vitamin mix S3***		1 ml.		1 ml.	
PII Metals****	1.0 ml.	3 ml.		3 ml.	1 ml.
SII Metals†					1 ml.
P8 Metals††			1 ml.		
Tris buffer	0.1 g.	0.1 g.	0.1 g.	0.1 g.	0.1 g.
Nitrilotriacetic acid		(10 mg.)		7 mg.	(10 mg.)
pH	7.6	7.8	7.4–7.6	7.8–8.0	7.8–8.0

* Developed by L. Provasoli for tropical species of dinoflagellates.

** One ml. of Vitamin mix 8 contains: thiamine HCl, 0.2 mg.; nicotinic acid, 0.1 mg.; putrescine 2HCl, 0.04 mg.; Ca pantothenate, 0.1 mg.; riboflavin, 5 μg.; pyridoxine 2HC1, 0.04 mg.; pyridoxamine 2HCl, 0.02 mg.; *p*-aminobenzoic acid, 0.01 mg.; biotin, 0.5 μg.; choline H citrate, 0.5 mg.; inositol, 1.0 mg.; thymine, 0.8 mg.; orotic acid, 0.26 mg.; B_{12}, 0.05 μg.; folic acid, 2.5 μg.; folinic acid, 0.2 μg.

*** One ml. of Vitamin mix S3 contains: thiamine HCl, 0.05 mg.; nicotinic acid, 0.01 mg.; Ca pantothenate, 0.01 mg.; *p*-aminobenzoic acid, 1 μg.; biotin, 0.1 μg.; inositol, 0.5 mg.; folic acid, 0.2 μg.; thymine 0.3 mg.

**** One ml. of PII metal contains: ethylenediamine tetracetic acid, 1 mg.; Fe (as Cl), 0.01 mg.; B (as H_3BO_3), 0.2 mg.; Mn (as Cl) 0.04 mg.; Zn (as Cl), 0.005 mg.; Co (as Cl), 0.001 mg.

† One ml. of SII metals contains: Br (as Na), 1.0 mg.; Sr (as Cl), 0.2 mg.; Rb (as Cl), 0.02 mg.; Li (as Cl), 0.02 mg.; I (as K), 0.001 mg.; Mo (as Na), 0.05 mg.

†† One ml. of P8 metal contains: Na_3 versenol, 3 mg.; Fe (as Cl), 0.2 mg.; Mn (as Cl), 0.1 mg.; Zn (as Cl), 0.05 mg.; Co (as Cl) 0.001 mg.; Cu (as Cl), 0.002 mg.; Mo (as Na), 0.05mg.; B (as H_3BO_4), 0.2 mg. Versenol = hydroxyethyl-ethylenediamine triacetic acid.

ASP2, ASP2NTA, ASP6, ASP7, ASP12, ASP12NTA and D; Table II). *Conchocelis* growth was obtained in most of these media except ASW9 and ASP2. ASP1, ASP6, ASP12NTA, ASP12 and ASW8 gave very good growth; ASP7, D, and SWII were less good; SWI very poor.

Young germlings of *P. tenera* (5 mm. long) cultured at 14–16° C., and illuminated 13 hours a day with 400–500 foot-candles of incandescent light, did not grow normally (see later) and produced carpospores from which *Conchocelis* colonies developed.

Suitable media and cultural conditions for free-living growth of Conchocelis

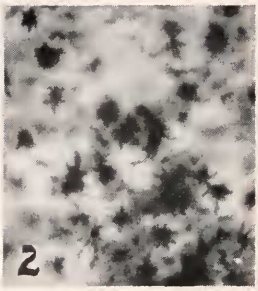
Several artificial media and enriched sea waters permit continued growth of the *Conchocelis* phase. In decreasing order, ASP12NTA, ASP2NTA, ASP12, ASP6, and ASP7 are the most suitable artificial media, and SWII and SWI the enriched sea waters. *Conchocelis* cultures easily last 6 months; the color of the colonies varies in different media: pinkish-red in ASP12NTA, ASP12, and ASP6; pale brown in ASP2NTA, dark brown in SWII, and pinkish-grey in ASM. The color is more intense in the center of the colony, probably because of the presence there of intensely pigmented monosporangial branches. The type of medium influences growth rate and monosporangia formation. In decreasing order, growth was fastest in ASP12NTA, SWII and ASP7 and slower in ASP2, ASP12, and MEC3. Monosporangia were formed and monospores liberated earlier in ASP12NTA, and in decreasing order in ASP12, ASP7, ASP2, SWII, SWI. The temperature range is between 10 and 26° C.; the optimum between 13 and 20°. Single pieces of the filament of the *Conchocelis* phase (~1 mm.) transferred in new media grew into new *Conchocelis* colonies vegetatively. It was possible in this way to subculture the *Conchocelis* phase: 5 serial transfers (one every 2–3 months from February to December, 1960) resulted in good growth. Quite likely the *Conchocelis* phase can be grown indefinitely as free-floating colonies in liquid media.

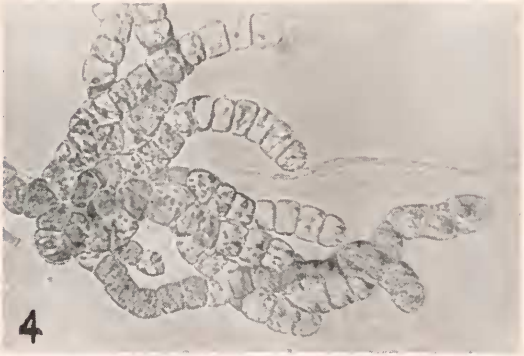
The *Conchocelis* colonies in test tubes of liquid media generally grew at the bottom of the tube attached to the glass wall and appeared as fuzzy balls 4–10 mm. in diameter (Fig. 1). In larger containers, where they grow free-floating in the medium, they were stellate, often reaching a diameter of 10–15 mm. (Fig. 2). The *Conchocelis* phase can be grown, but poorly, also on agar slants in screw-cap tubes.

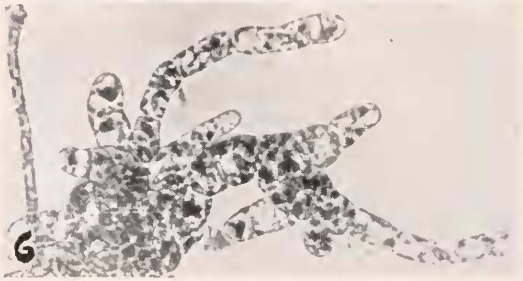
At the beginning of this work the free-living *Conchocelis* colonies were grown in subdued light (20–40 ft.c.) to simulate natural conditions; under these conditions growth was quite slow. Later, in surveying the effect of light intensity, it was found that growth was greatly increased by higher light intensities—the higher, the better (maximum tried, 350 ft. c.). Incandescent and fluorescent light were equally effective; however, the color of the *Conchocelis* was different: reddish in fluorescent light and cool brown-black in incandescent light. Continuous illumination also favored growth. Under these conditions (350 ft. c. continuous fluorescent light) mass cultures were obtained in 2-liter Erlenmeyer flasks and in tall, 4-liter bottles (Fig. 3) by gradual transfer in increasingly larger containers (10 ml. inoculated into 100 ml.; 100 ml. in 1 liter, etc.).

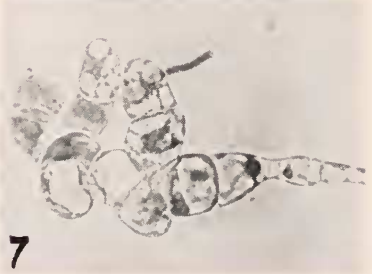
Effect of photoperiodism on monosporangia and monospore production

Kurogi's experiments (1959) indicated that photoperiodism may govern monosporangia production and monospore liberation in *Conchocelis* grown in shells. The following experiment was set to test the effect of photoperiodism on free-living *Conchocelis*. With fluorescent light of 150–250 ft. c., a daily photoperiod of 8–11 hours induced formation of monosporangia in 2–3 weeks and young thalli in 3–8 weeks from the time of inoculation into new media of pieces of *Conchocelis* filaments (Figs. 5, 6, 7). No substantial difference was found in cultures grown









FIGURES 1–7, 12.

at 13–15° C. or 18–20° C. in high light (150–250 ft. c.) for a daily photoperiod of 8–11 hours. When the light intensity was reduced to 30–50 ft. c., the appearance of young thallus germlings was greatly retarded in the 8-hour photoperiod (> 96 and < 184 days) and apparently prevented in the 11-hour photoperiod (no leafy thalli in 180–240 days).

Under continuous fluorescent light at intensities of 150–250, 60–100, and 10–20 ft. c., both at 13–15° and 20–26°, neither spores nor thallus germlings were found during the two experiments which lasted, respectively, 180 and 240 days. Growth of *Conchocelis* filaments and colonies proceeded normally, and may, indeed, be favored by continuous light; very good mass cultures were obtained in continuous light. Intensely purple, inflated portions similar to monosporangia, appeared after a month or more in cultures of 60–250 ft. c. At that time these structures were thought to be small monosporangia. Unfortunately the observations of these experiments were done at great intervals (one month or more), and through the walls of the test tubes using a dissecting microscope. Only later, when it became evident that these structures were not producing spores, was a simple experiment tried: a *Conchocelis* colony grown for two months in continuous light at 13–15° C. was transferred to new medium and illuminated 8 hours a day; after 5 weeks many thallus germlings (5–8 mm. long) were growing alongside the *Conchocelis* colony.

Evidently, maturation of monosporangia, release of monospores, or both, are induced by a short photoperiod and prevented by continuous light.

The sporangia produced in continuous light (250–350 ft. c.) in the mass cultures seemed to be morphologically different from the monosporangia produced under short-day conditions. Sporangia cells in continuous light have thicker walls and length of the cells is usually about half their diameter; some cells are quadrate (Fig. 4). They are very similar to the "plantlets" described for *P. umbilicalis* var. *laciniata* by Drew (1954, p. 203, Fig. 4c).

On the contrary, the cells of the short-day monosporangia often have elongated cells and the appearance of monosporangia is much more twisted (Figs. 5, 6, 7) because of the lateral branches. Are the continuous-light sporangia undeveloped or abnormal monosporangia, or are they a new type of sporangium? The evidence at hand does not exclude either possibility.

The aforementioned experiment of transferring a *Conchocelis* colony from continuous light to short-day is indicative but not conclusive; only one observation was made 38 days after the transfer: young thalli of 5–8 mm. were found. The formation *de novo* of true monosporangia is not excluded because under the same light and temperature conditions (exp. *II*, Table III) young thalli appeared between 22 and 31 days in a culture started with pieces of *Conchocelis* filaments (no length was noted in the protocols; they were probably 2–3 mm. long).

FIGURE 1. Colonies of free-living *Conchocelis* in artificial medium.
FIGURE 2. Free-floating *Conchocelis* (detail of Figure 3).
FIGURE 3. Mass culture of *Conchocelis* in aerated 3-liter bottle, continuous light.
FIGURE 4. Sporangia formed in continuous illumination.
FIGURE 5. Typical monosporangia formed in short day conditions (8–11 hours daily).
FIGURES 6, 7. Same detail.
FIGURE 12. Young thallus germlings, and monospores.

The hypothesis that the *Conchocelis* phase can produce other sporangia besides the monosporangia has already been advanced by Drew for *Porphyra umbilicalis* var. *laciniata* (1954) and *Bangia fuscopurpurea* (1958) to explain *Conchocelis* infections in sterile shell derived from other *Conchocelis*-infected shells. This possibility is greatly reinforced by our experiments with *P. tenera*. More than 5 serial transfers were done directly from *Conchocelis* to *Conchocelis* in test tubes or in mass culture without passing through the thallus phase or carpospores: in every case, inoculating pieces (in test tubes) of *Conchocelis* filaments or entire colonies (in mass cultures) led to numerous new colonies. These experiments also do not prove conclusively that the increase in the number of *Conchocelis* colonies is due to the production of special spores developing into new *Conchocelis* colonies,

TABLE III

Effects of short-day and continuous light conditions on Conchocelis *phase**

Temperature	Light** period	Light intensity**	Appearance of sporangia†	Appearance of foliaceous thalli†	Remarks
13–15° C.	8 hr.	150–250 ft. c.	*I* 19 days *M.*	<46 days	good growth of thalli (1–1.5 cm.)
			II <22 days *M.*	>22–<31 days	3 cm. thalli in 96 days
		30–50 ft. c.	*I* 27 days *M.*	none up to 84 days	discontinued at 84 days
			II <48 days *M.*	>96–<184 days	small thalli
18–20° C.	11 hr.	150–250 ft. c.	*I* 19 days *M.*	>56–<84 days	good growth of *Conchocelis*-thalli soon bleached
			II 23 days *M.*	31 days	
		30–50 ft. c.	*I* 19 days *S2*	none up to 240 days	
			II 23–31 days *S2*	none up to 184 days	large *Conchocelis* colonies
13–15° C.	continuous	150–250 ft. c.	*I* 35 days *S1–S2*	none up to 240 days	††
			II 31 days *S1–S2*	none up to 184 days	
		30–50 ft. c.	*I* 84 days *S2*	none up to 240 days	
			II 72–96 days *S2*	none up to 184 days	
20–26° C.	continuous	60–100 ft. c.	*I* 35 days *S2*	none up to 240 days	
			II 31 days *S2*	none up to 184 days	
		10–20 ft. c.	*I* 56 days *S2*	none up to 240 days	(Figs. 8–11)
			II *S2*	none up to 184 days	

* Media: ASP7 and SWII. Results of two separate experiments (*I* and *II*). *M* = monosporangia; *S1*, see Figure 4; *S2*, see Figures 8–11.
** Fluorescent: "cool white."
† Days from date of inoculation. Inoculum = a small piece of *Conchocelis* filament.
†† At 50 days a *Conchocelis* colony was transferred to new medium and to 8 hours of light. In a month monospores and thalli appeared.

because even small pieces of *Conchocelis* filament, which can grow into a full new colony, could have been present. However, the short-celled sporangia produced by *P. tenera* in continuous light (Fig. 4) or the sporangia-like swollen cells described by Drew for *Bangia* (1958, Fig. 3, p. 366) may be a new type of sporangium whose spores produce a new *Conchocelis* colony.

Other very strange structures are produced in continuous (10–100 ft. c.) or 11 hours subdued fluorescent light (30–50 ft. c.) at 13–15° C. and 20–26° C. (Table III). The similarity of the latter sporangia with fungal structures is striking (Figs. 8, 9, 10, 11; sporangia (?) *S2* of Table III).

The variety of structures created in different lights and temperatures shows that *P. tenera* has unusual powers of adaptation. The *Conchocelis* phase can now

be grown free, making the morphological observations easy. This permits a wider analysis of the unusual morphological versatility of *P. tenera* as well as of the possible deviations from the normal life-cycle induced by various lights and temperatures.

The Leafy Thallus Phase

Some cultural conditions for the growth of the thallus had been determined previously (Iwasaki and Matsudaira, 1958, and unpublished).

(1) Leafy thalli grow normally in enriched sea water (Miquel's sea water), while they are short and unhealthy when grown in filtered, unenriched inshore sea water.

(2) High-intensity, incandescent light is required for normal continued growth; growth is, however, slower than in natural sunlight. Young plants grown in fluorescent light die in a few days.

(3) Young plants grow normally when illuminated 8–10 hours daily but die quickly when grown in continuous light.

These results were on the whole confirmed by the present investigation.

Effect of media on leafy thallus growth

Thalli (1–2 mm.) derived from monospores produced by free-living *Conchocelis* (Fig. 12) were grown in enriched sea water and artificial media at 14–16° C.,

Table IV

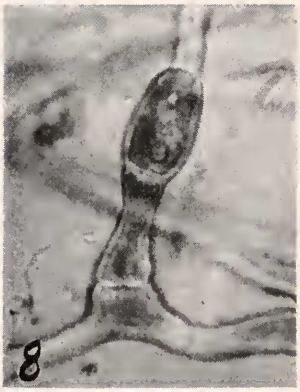
Thallus growth (two-month)

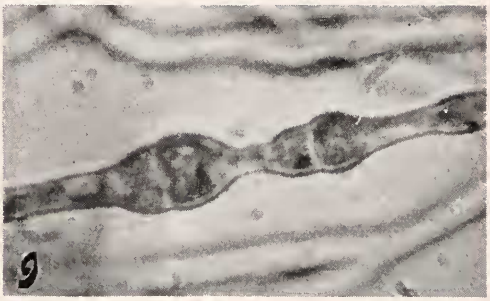
Media	Growth	Color
ASP1	10 × 40 mm.	brown
ASP2	8 × 50 mm.	
ASP12	20 × 40 mm.	red-brown
ASP12NTA	8 × 20 mm.	
SWI	8 × 80 mm.	reddish
SWII	20 × 35 mm.	pale brown

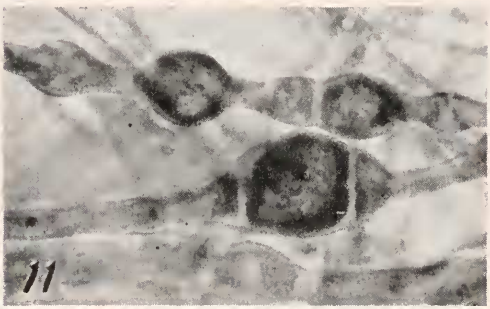
and illuminated 9 hours daily with 400 ft. c. of incandescent light. The experiment was done in test tubes (20 × 120 mm.) containing 10 ml. of medium; once a month the medium was replaced aseptically with 10 ml. of fresh medium. Good normal growth was obtained in two months in some artificial media and in enriched sea water (Table IV and Fig. 13). Narrow long thalli were obtained in SWI and most artificial media, broader thalli in SWII and ASP12. The 1957 experiments were done during the season in which the thalli grow in nature (fall-winter). On the contrary, the new experiment was done between May and August, 1960, indicating that normal thalli can be grown out of season if the light period is suitable (8–11 hours daily).

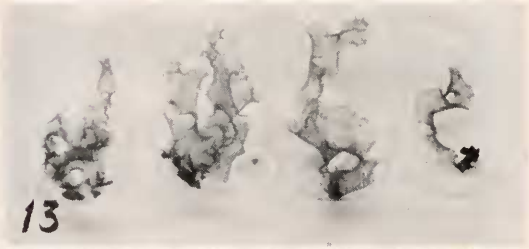
Effect of long-day conditions on leafy thalli

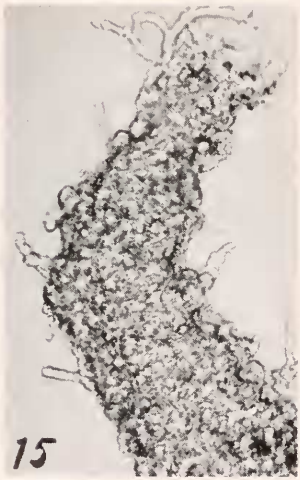
In retrospect, the importance of the photoperiod for *Porphyra tenera* might have been suspected because the two phases of the life-cycle of *P. tenera* correspond so sharply to the seasons. The leafy thallus grows in the short-day seasons











FIGURES 8, 9, 10, 11. Inflated cells (sporangia?) produced in subdued light.

FIGURE 13. Two-month-old thalli grown in test tube. From left, medium SWI, SWII, ASP12, ASP1.

FIGURE 14. Young thallus degenerated under long-day conditions (13 hours daily). Lower part bleached; large pigmented cells at top; *Conchocelis* filaments germinating from "spores."

FIGURE 15. Root-like projections growing out of a young thallus grown in SWII under long-day conditions.

(autumn-winter), the *Conchocelis* phase in the long-day seasons (spring-summer). Furthermore, the transition between the two phases of the life-cycle coincides with the equinox (Fig. 16). On the contrary a great part of the temperature range (7–21° C.) is common to the two phases: normal thalli grow in nature between 3–21° C. and the *Conchocelis* between 7 and 25° C. Therefore, only the lower zone (3–7° C.) may be suspected to affect *Conchocelis* growth and the upper zone (21–25° C.) thallus growth. These considerations, and the already known effects of continuous light on thallus growth (Iwasaki and Matsudaira, 1958) and short day on monosporangia formation (Kurogi, 1959) suggested trial of growth under long-day conditions.

Five young germlings (0.5 mm.), derived from monospores of free-living *Conchocelis*, were inoculated in each tube of the following media: SWI, SWII, ASP1, ASP2, and ASP12. They were incubated at 14–16° C. and illuminated 13 hours daily with 400–500 ft. c. of incandescent light. The controls were grown

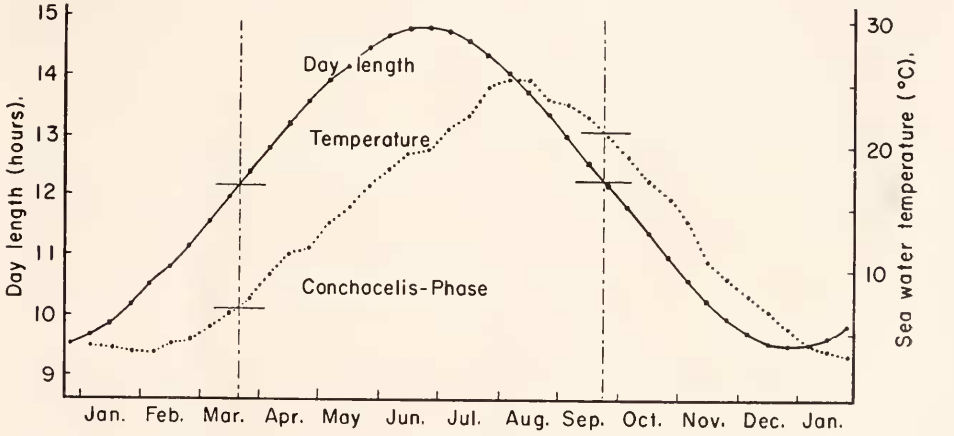


FIGURE 16. Day length at Sendai and average sea water temperature in Matsushima Bay.

under similar conditions but illuminated 8 hours daily. The control gave, as in the previous experiment, normal thalli of the narrow shape.

The thalli in long-day conditions grew very slowly and very soon became thick and irregular in shape. After 20 days or more, the thalli became pale, leaving numerous scattered big reddish-colored cells. The thallus around the edges assumed the appearance of a callus tissue (Fig. 14). After 40 days (in ASP2) or more, spores were released. Since these spores germinated into filaments which later formed well-developed *Conchocelis* colonies, we assume that, at least functionally, they are equivalent to carpospores. These events differ slightly in time and amount of growth in the various media except SWII. In SWII the thallus after 27 days started to produce root-like projections (Fig. 15) which branched out into thinner filaments; after two months the thallus became covered with *Conchocelis* colonies. Apparently 13 hours of daylight, which corresponds at the latitude of Sendai (39° N) to late April, already inhibits normal thallus growth and in-

duces the formation of structures functionally equivalent to carpospores. This experiment was repeated later with similar results.

In another experiment in tall covered containers (10 cm. diameter; 7 cm. high) containing 200 ml. of ASP1, young thallus buds (1–2 mm.) were grown for one month (April 22–May 25) at 14–16° and illuminated 8 hours daily with 400 ft. c. of incandescent light: the thalli, which had reached by then an average size of 3 cm.^{2}, were illuminated for 10 days (May 25–June 4) with 100–200 ft. c. of incandescent light: on alternate days, 8 hours daily followed by one day in continuous light. After this period of alternating photoperiods, the culture was grown in 100–200 ft. c. and 8 hours daily of fluorescent light. In a month (July 5) big, dark cells appeared, scattered at the edges of the leafy thalli which were stunted and curled. Ten days later these cells produced germ tubes which developed into *Conchocelis* colonies. Two months (August 5) after the alternating light treatment, many colonies of *Conchocelis* were growing free on the bottom of the dish and covering the stunted disintegrating thalli. After 82 days (August 25) mature monosporangia were formed and a few days later the monospores were released and produced thallus germlings. These germlings on September 10 had already reached an average size of 1 cm.^{2}

The complete life-cycle was obtained in 5–6 months.

It is remarkable that the leafy thalli of the second generation grew normally, though slowly, in fluorescent light and at low intensities (100–200 ft. c.). This is by no means an isolated case of thallus growth in fluorescent light: all the cultures of *Conchocelis* grown at 13–20° C. for 8–11 hours daily of fluorescent light eventually released monospores (1–4 months, depending upon light intensity). These spores gave rise to leafy thalli reaching 2–10 mm. before they became pale and died. The arrest in growth of these leafy thalli was probably due to lack of nutrients: the medium in these experiments was not changed monthly, as was done for the experiment on thallus growth in different media. Ability of the thalli to grow in fluorescent light may be an adaptation to utilization of fluorescent light acquired during the *Conchocelis* phase: the *Conchocelis* phase does not require incandescent light. This adaptability, whatever the cause, reflects again the great plasticity and versatility of *P. tenera*.

Discussion

These results may help solve some of the problems of life-cycle and growth potencies of *P. tenera*. Solutions here, in turn, may improve the farming of this sea weed. The first report of the entire life-cycle of a *Porphyra* obtained *in vitro* is the one of Hollenberg (1958). He obtained from carpospores *Conchocelis*-like filaments which formed sporangia and liberated spores (16 days from carpospore germination). These spores in turn developed into blade-like plantlets (young thalli). Since the cultures were grown during the summer in north light, it is possible that the very rapid formation of sporangia and the poor growth of the *Conchocelis* phase were due to light conditions. While this paper was being written, the paper on the *Conchocelis* stage of *P. umbilicalis* by Kornmann (1960) appeared. Like us, Kornmann obtained the complete life-cycle *in vitro*. He started in November, 1959, with a "plantlet" (probably an immature, or abnormal monosporangium) cultured in Erdschreiber. The "plantlet" became fertile and made

monospores which did not develop. Only a few cells of this structure remained vegetative and reproduced in a month another "plantlet" (without "rhizoids") which produced many monospores. Of these, only 6 germinated into leafy thalli which in a month and a half reached 1.5–2 mm. in length. The thalli formed "Bällchen" from which thin filaments grew out; the filaments, by division, produced "zweige" (his Figure 5 = "plantlet" = monosporangia?). From the "zweige" arose as side-branches ("seitliche Verzweigung") thin filaments which in free culture produced a confused ball of yarn (verworrene Knäuel = free *Conchocelis*) or enveloped the original plantlet. These filaments grow also as a typical *Conchocelis* in calcareous shells.

Unfortunately, no data are given of the light period under which the cultures were grown. From his Figures 2 and 5, the "plantlets" are very similar to monosporangia. If so, the thin filaments (which are *Conchocelis* filaments) should produce, and not be produced by the monosporangia (as Kornmann states in Figure 5 and the text). But the structure in Figure 5 could be equivalent to the sporangia which were produced in our *Conchocelis* colonies grown in continuous light (our Fig. 4). As mentioned, these sporangia are suspected of producing spores germinating into a new *Conchocelis*. Kornmann's light conditions seem also to be inadequate for thallus growth because, as in our thallus cultures under long-day conditions, *Conchocelis* filaments arise from the thallus (Kornmann, Fig. 3B) or big colored cells are formed (Kornmann's "Bällchen" which can be seen at the base of the thallus of Figure 3, C) from which *Conchocelis* filaments arise. Kornmann's Figure 1C represents, most likely, true monosporangia and *Conchocelis* filaments.

Whatever the interpretation, it is seen that, both in Kornmann's and in our experiments, the life-cycle can be obtained *in vitro*. Detailed morphological studies are planned to solve some of the many questions; *e.g.,* what is the typical morphology of the true monosporangia of *Conchocelis* grown free—how do they differ from those produced in shells? What are the mysterious "plantlets" of Drew, and of Figures 1A and 2A of Kornmann—are they sporangia whose spores develop another *Conchocelis* phase, or abnormal monosporangia? What are the deviations from the natural life-cycle in shells that develop when the *Conchocelis* phase is grown free and in different day-lengths and light-intensities? What are the big, dark cells formed in the degenerating thalli under long-day conditions?

The present research confirms and extends previous results on the effect of the photoperiod on *P. tenera*. As mentioned, the *Conchocelis* phase grows in nature during the long-day seasons and the leafy thallus phase in short-day seasons. The leafy thallus phase is apparently a short-day plant: growth is arrested and the thallus degenerates when exposed to 13 hours of light daily. The *Conchocelis* phase is not strictly a long-day plant: *in vitro* it grows, but slowly, under short-day (8-hour) conditions and in subdued light. However, high light, longer day (11-hour), and especially continuous light enhance growth vigorously. The incomplete data available indicate that the photoperiod governs the formation of monosporangia and the liberation of monospores. Our *in vitro* experiments confirm fully the results of Kurogi (1959) obtained with *Conchocelis* grown in shells. He found that photoperiods of 10 and 12 hours of light (corresponding to conditions of winter, spring and autumn, respectively) induce an abundant formation of monospores, while 15 hours of light daily did not enhance the formation of monosporangia.

Furthermore, the *Conchocelis* which were liberating monospores in 10-hour photoperiods continued for only a few days, and then stopped liberating monospores, when transferred to 15 hours of light; conversely the long-day (15-hour) *Conchocelis* began to liberate monospores after they were transferred to short-day (10-hour) conditions. Similarly the *in vitro* experiments on free-living *Conchocelis* show that short-day (8-, 11-hour) induces early formation of monosporangia and liberation of monospores. Continuous light, or subdued 11-hour photoperiods, induce the formation of interesting and different sporangia, or peculiar inflated cells in the *Conchocelis* filaments, whose fate and origin need further investigation.

The preliminary experiments on the thallus indicate that the photoperiod also governs the formation of carpospores; 13 hours of light daily induce cessation of growth and degeneration of the leafy thallus, followed by formation of carpospores or their physiological equivalents. Exposure of full-grown thalli to different photoperiods is now needed to define precisely the effect of the photoperiod on carpospore production.

These findings emphasize the need of determining the effect of photoperiods on the life-cycle and alternation of generations in sea weeds. Föyn (1955) had observed that the northern species of *Ulva* (*lactuca*) can grow normally in continuous light, while the southern Mediterranean species (*Thureti*) dies in such conditions.

This work was supported by contract NR 104–202 of the Office of Naval Research and by Grant G–1198 of the National Science Foundation to Dr. L. Provasoli, of Haskins Laboratories. I wish to thank Dr. Provasoli for his hospitality, advice and constant interest.

Summary

1. The complete life-cycle of *Porphyra tenera* was obtained *in vitro*.

2. Chemically defined media or enriched sea water permit good growth of these unialgal (not bacteria-free) cultures.

3. Under suitable light and temperature, the complete life-cycle is completed in 5–6 months. Both the *Conchocelis* and the thallus phases may be grown out of season.

4. The *Conchocelis* phase grows well free in liquid media; a calcareous substrate is unnecessary. *Conchocelis* colonies grown in liquid media when free-floating, are stellate and round, but mold-like when attached to glass walls. They are brown-black or purple-red, depending on the composition of the medium. Rapid and abundant growth of the free *Conchocelis* is elicited by high-light intensities. Fluorescent light is a good light source.

5. Monosporangia formation and release of fertile monospores are induced by short-day conditions (8–11 hours daily); monosporangia and germinating monospores develop after 1–2 months from the inoculation of the *Conchocelis* filaments. In continuous light, *Conchocelis* growth is rapid but the sporangia produced are somehow different from the ones produced in short-day conditions.

6. In continuous light, the number of colonies increases rapidly after transfer to new media. This could be due to formation of new colonies from small pieces of filaments. However, even though free spores were not found, it is not excluded that new *Conchocelis* colonies may have been derived from special spores.

7. The *Conchocelis* phase was cultured for one year by transferring free *Conchocelis* colonies or pieces of filaments every two months in new media. Mass cultures with good yields were obtained in continuous fluorescent light.

8. The leafy thallus, derived from monospores grown in shells, grows well and normally in artificial media, at 13–18° C. and in high intensity incandescent light of 8–11 hours daily, but not in fluorescent light.

9. A photoperiod of 13 hours daily inhibits growth of young thalli (1–2 mm.). The thalli became thick, curly, degenerate, assume a callus appearance, bleach almost completely except for scattered groups of dark-pigmented, big cells which produce spores germinating into *Conchocelis* filaments. In one type of enriched sea water (SWII), the thalli, after thickening, and while degenerating, produce rhizoid-like structures which give rise to *Conchocelis* filaments.

10. In nature, the *Conchocelis* phase grows in the long-day seasons, the leafy thallus phase grows in the short-day seasons; and the transition between the two phases is almost exactly at the equinox. On the contrary, no correlations exist between temperature and the phases of the life-cycle: a large temperature zone (7–21° C.) is common to the two phases. Similarly, our preliminary experiments show that the length of the photoperiod has remarkable effects on the *Conchocelis* and leafy-thallus phases of *P. tenera*. The photoperiod governs, besides growth, the formation of the spores producing the next phase of the life-cycle. It is reasonable, therefore, to suppose that like land plants, some sea weeds, or phases of their life-cycle, may be long- or short-day plants.

LITERATURE CITED

Drew, K. D., 1949. *Conchocelis* in the life history of *Porphyra umbilicalis* (L.) Kütz. *Nature*, **164**: 748.

Drew, K. D., 1954. Studies in the Bangioidae III. The life-history of *Porphyra umbilicalis* (L.) Kütz. var. *laciniata* (Lightf.) J. Ag. *Ann. Bot. N. S.*, **18**: 183–211.

Drew, K. D., 1958. Studies in the Bangiophycidae IV. The *Conchocelis*-phase of *Bangia fuscopurpurea* (Dillw.) Lyngbye in culture. *Pubbl. Staz. Zool. Napoli*, **30**: 358–372.

Föyn, B., 1955. Specific differences between northern and southern European populations of the green alga *Ulva lactuca* L. *Pubbl. Staz. Zool. Napoli*, **27**: 261–270.

Hollenberg, G. J., 1958. Culture studies of marine algae III. *Porphyra perforata*. *Amer. J. Bot.*, **45**: 653–656.

Iwasaki, H., and C. Matsudaira, 1958. Culture of a laver, *Porphyra tenera* Kjellm. I. Preliminary research on cultural conditions. *Bull. Jap. Soc. Sci. Fish.*, **24**: 398–401

Kornmann, P., 1960. Von *Conchocelis* zu *Porphyra*. *Helgolander Wiss. Meeresunters.*, **7**: 189–193.

Kurogi, M., 1953. Studies of the life-history of *Porphyra*. 1. The germination and development of carpospores. *Bull. Tohoku Reg. Fish. Lab.*, **No. 2**: 67–103.

Kurogi, M., 1959. Influences of light on the growth and maturation of *Conchocelis*-thallus of *Porphyra*. I. Effect of photoperiod on the formation of monosporangia and liberation of monospores. *Bull. Tohoku Reg. Fish. Lab.* **No. 15**: 33–42.

Kurogi, M., and K. Hirano, 1956. Influence of water temperature on the growth, formation of monosporangia and monospore-liberation in the *Conchocelis* phase of *Porphyra tenera* Kjellm. *Bull. Tohoku Reg. Fish. Res. Lab.*, **No. 8**: 45–61.

Tseng, C. K., and T. J. Chang, 1954. Studies on the life history of *Porphyra tenera* Kjellm. *Sci. Sinica*, **4**: 375–398.